

RE-IDENTIFICATION OF AN EXOTIC BEE INTRODUCED TO THE HUNTER VALLEY REGION, NEW SOUTH WALES – *SELADONIA HOTONI* (VACHAL, 1903) (HYMENOPTERA: HALICTIDAE)

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Abstract

In 2004 and 2006, well-established populations of an exotic halictine bee were found in the Upper Hunter Valley region of New South Wales, Australia. On the basis of morphology, the species was identified as *Halictus (Seladonia) smaragdulus* Vachal, 1895 by an expert familiar with that genus. Subsequently, it was discovered that there are six species in the *S. smaragdula* complex, but none of the six had the same mitochondrial DNA barcode as the species found in Australia. The introduced bee has been shown to be conspecific with an African species by mitochondrial COI DNA sequences and identified as *Seladonia hotoni* (Vachal, 1903) by morphology.

Introduction

The initial report of a small, metallic green exotic bee in the Hunter Valley (Gollan *et al.* 2008) identified it as belonging to the western Palaearctic species *Halictus (Seladonia) smaragdulus* Vachal, 1895. Accepting the raising of *Seladonia* Robertson to generic level (Pesenko 1999), the name became *Seladonia smaragdula* (Vachal) Identification was based on the external morphology of specimens sent to A. W. Ebmer, an expert in *Seladonia* taxonomy.

Other features of the male genitalia, however, indicated that *S. smaragdula* was in fact a species complex (Pauly and Rassel 1982), which led the present authors to exchange images, comparison of which suggested that the bees in Australia might belong to one of the other species in the complex. Subsequently, molecular evidence was shown to support the idea of a species complex (Schmidt *et al.* 2015), which now includes *S. smaragdula* and five additional cryptic species (Pauly *et al.* 2015).

Comparison of DNA barcoding sequences of Australian specimens with those of a member of the *S. smaragdula* complex with similar male genitalia (*S. orientana* Pauly & Devalez, 2015), showed that the species were different (*vide infra*). Indeed, the species introduced to Australia was none of the six in the *S. smaragdula* complex. We report here that the introduced species has been shown by morphological and molecular evidence to be the southern African species *Seladonia hotoni* (Vachal, 1903).

DNA barcoding

DNA was extracted from an African *S. hotoni* specimen and sequenced as described previously (Pauly *et al.* 2015). Australian material was treated similarly, but using the DNeasy Blood and Tissue Extraction kit distributed by QIAGEN Pty Ltd and the ExoSAP-IT PCR purification system (USB Corporation). Partial mitochondrial COI sequences (658 bp) were obtained from 36 Australian specimens collected at twelve different locations and a South African specimen collected at Knersvlakte, along Gemsbokrivier Pad, in Western Cape Province (*leg.* M. Kuhlmann).

The COI sequences for the Australian specimens contained two haplotypes with a divergence of 0.5% in roughly equal proportions (20 type 1, 16 type 2). All but one of the differences between the sequences were in the third codon position. Both haplotypes differed from that of *S. hotoni* from Africa by less than 1% (Table 1), whereas they differed from those of the *S. smaragdula* complex by more than 7%. Interspecific differences between species in the *S. smaragdula* complex are between 3% and 6%, while intraspecific variation ranges from 0.2% to 2.7% (Pauly *et al.* 2015).

Table 1. Divergences (number of substitutions/length of sequence) between haplotypes of *Seladonia hotoni* from Africa and Australia (GenBank accession numbers KX360229-31) and other *Seladonia* species available in GenBank (KT601640-KT601694).

	<i>S. hotoni</i> (Africa)	<i>S. orientana</i>	<i>S. smaragdula</i> species complex
<i>S. hotoni</i> (Australia)	0.006-0.008	0.084-0.096	0.073-0.135

There was one non-synonymous difference between the two main Australian haplotypes, which corresponds to interchange of valine and isoleucine in the translated protein. Exchange of these hydrophobic amino acids is expected to have a relatively small effect on the protein, but was nevertheless unexpected.

Both the Australian haplotypes were found at 6 of 8 places where more than one specimen was collected. The uniform distribution of the haplotypes and the divergence between them is evidence that the introduction involved more than one individual. *Seladonia hotoni* is known to nest in the ground and nests have been found at Muswellbrook and Sans Souci. One possibility, therefore, is that the bees might have arrived in nesting tunnels in soil.

Morphology

Seladonia hotoni is very similar to *S. smaragdula sens. lat.* and the two can be easily confused when their geographic origin is unknown. The holotype of *S. hotoni* has been examined (by AP) and, while the species cannot be distinguished from *S. submediterranea* Pauly, 2015 or *S. orientana* using the male genitalia, it does differ in other subtle characters. *Seladonia hotoni* has

a shorter head (length/width: female = 0.91, s.d. 0.02, n 25; male = 0.98, s.d. 0.02, n 14) and small differences in surface sculpture. The African species of *Seladonia* have been catalogued by Pauly (2008). They are now illustrated and mapped on the website Atlas Hymenoptera (Pauly 2016).

Predicted distribution of *Seladonia hotoni* in Australia

The introduced species was surveyed between October 2008 and February 2010. In order to determine the potential extent of the incursion and guide further surveys, predicted distributions were calculated using various combinations of Worldclim predictors and the known distribution of *S. smaragdula sens. lat.* (Ashcroft *et al.* 2012). Irrespective of the merits of any particular model, it was of interest to know whether the re-identification altered the predicted distribution of the introduced species. Figure 1 compares predictions for the two groups calculated with Maxent version 3.3.3k with default values for regularisation parameters (Phillips *et al.* 2006) using four commonly used Worldclim parameters: annual mean temperature; annual precipitation; maximum temperature of warmest month and minimum temperature of coldest month. It showed that *S. hotoni* has a higher probability than *S. smaragdula* of colonising southern areas of Australia.

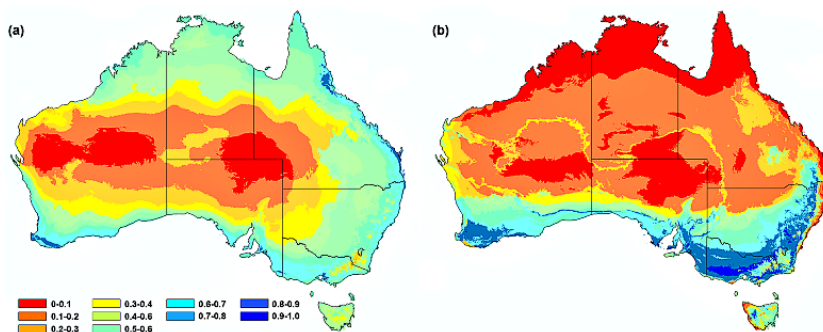


Fig. 1. Logistic output from Maxent using four commonly used predictors for (a) *S. smaragdula sens. lat.* and (b) *S. hotoni*.

New survey and conclusion

A brief survey was conducted on 5 and 6 December 2015 to determine whether the bee was still present in the Hunter region. Ten sites from which the bee had previously been collected were visited and *S. hotoni* specimens were taken at 7 of those sites. Few flowers of any kind is one reason for its absence at the other three sites. Perhaps unsurprisingly, high densities of flowers, especially the introduced species *Galenia pubescens* (Aizoaceae), were associated with many of the collections when sweeping. While there are no specific flower visiting records for *S. hotoni* in Africa, small *Seladonia* species are frequently found on *Galenia sarcophylla* (M. Kuhlmann pers. comm.), which is widespread in the southwestern corner of the continent, an

area that covers about half the known range of *S. hotoni*. No reports have been received of the species spreading to new areas but, equally, no systematic surveys have been performed.

The genus *Seladonia* contains a number of species that can be difficult to distinguish. Correct identification of the species introduced to Australia has required detailed morphological study of species within the genus (Pauly *et al.* 2015), supported by molecular barcoding. The revised identity is consistent with a higher predicted suitability of the Hunter Valley region. Nevertheless, our previous conclusion (Ashcroft *et al.* 2012) that suitable nesting sites may be just as important as climatic variables remains. The presence of two distinct mitochondrial haplotypes throughout the introduced population means that the introduction included more than one individual.

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